

SEQUENCE LISTING

<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 3fpo-07-05

<150> KR 2002-55575

<151> 2002 09 13

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<210> 1

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 1

<400> 1

ggctcttcag ccactccttt ggtgaag 27

<210> 2

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 2

2
gatcctc aggggtgac gat

23

3

27

DNA

Artificial Sequence

CALB primer 3

<400> 3

gatccgg ggtgacgat gccggag

27

<210> 4

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> GPD-err primer

<400> 4

gcagagctaa ccaataagg

19

<210> 5

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> T-0 primer

<400> 5

tgccagttgaa cacaaccac

19

<210> 6

<211> 1023

<212> DNA

<213> *Candida antarctica*

<220>

<221> sig_peptide

<222> (-51)..(-1)

<223> secretion signal

<400> 6

atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtac cgccactccc 9

ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 69

gatgcgggctc tgacctgcca ggggtgcttcg ccctcctcgg tctccaaacc catccttctc 129

gtccccggaa ccggcaccac aggtccaagc tegtctgact cgaactggat cccctctctc 189

gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgct caacgacacc 249

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 309

aacaagcttc ccgtgctcac ctgggtccag ggtgggtctgg ttgcacagtg gggctctgacc 369

ttcttcccca gtatcaggctc caaggctgat cgacttatgg cctttgcgcc cgactacaag 429

ggcacctgctc tcgcccggccc tctcgatgca ctgcgggtta gtgcaccctc cgtatggcag 489

caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gaccagatc 549
gtgcccacca ccaacctota ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 609
tcgccactcg actcactcta cctcttcaac gggaagaacg tccaggcaca ggctgtgtgt 669
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gactgcaacc ctcttccgc caatgatctg actcccgagc aaaaggctcg cgcggctgcg 849
ctcccggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 909
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ccc 972

<210> 7
<211> 1023
<212> DNA
<213> Candida antarctica

<220>
<221> sig_peptide
<222> (-51)..(-1)
<223> secretion signal

<400> 7
atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtag cgcactcct 9
ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 69
gatgcgggtc tgacctgcca aggtgcttgc ccatcctcgg tctccaaacc catccttctc 129

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct 189
gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc 249
cagggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 309
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg ggtctgacc 369
ttcttccccca gtatcaggtc caaggctgat cgacttatgg cctttgcgcc cgactacaag 429
ggcacgctcc tcgccggccc tctcgatgca ctccgggtta gtgcaccctc cgtatggcag 489
caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtggtct gaccagatc 549
gtgcccacca ccaacctcta ctccggcgacc gacgagatcg ttcagcctca ggtgtccaac 609
tcgccactcg actcatccta ccttttcaac ggaaagaacg tccaggcaca ggctgtgtgt 669
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ctcccggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 909
ctcatgccct acgcccgcct ctttgcagta ggcaaaagga cctgctccgg catcgtcacc 969
ccc 972

<210> 8
<211> 1023
<212> DNA
<213> Candida antarctica

<220>

<221> sig_peptide
<222> (-51)..(-1)
<223> secretion signal

<400> 8

atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtag cgccactcct 9
ttggtgaagc gtctgcttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 69
gatgcggggtc tgacctgcca ggggtgcttcg ccatactcgg tctccaaacc catccttctc 129
gtccccggaa cgggcaccac aggtccacag tcgttcgact cgaactggat cccctctctc 189
gcgtagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc 249
caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 309
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg ggggtctgacc 369
ttcttcccca gtatcaggtc caaggctgat cgacttatgg cctttgcgcc cgactacaag 429
ggcacctgac tcgccggccc tctcgatgca ctgcgggta gtgcaccctc cgtatggcag 489
caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gaccagatc 549
gtgcccacca ccaacctcta ctggcgacc gacgagatcg ttcagcctca ggtgtccaac 609
tcgccactcg actatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt 669
gggcccagc tcgtcatcga ccatacaggc tcgtcacct cgcagttctc ctacgtcgtc 729
ggtcgatccg ccttgcgtc caccacgggc caggctcgta gtgcagacta tggcattacg 789
gactgcaacc ctcttcccgc caatgatctg actcccagac aaaaggctgc cgcggctgctg 849
ctcctggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 909

ctcatgccct acgcccgcct ctttgcagta ggcaaaagga cctgctccgg catcgtcacc 969

ccc

972

<210> 9
<211> 341
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (-24)..(-8)
<223> secretion signal

<400> 9
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr

76

81

86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
91 96 101

Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
106 111 116

Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
126 131 136

Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
201 206 211 216

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
 281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
 301 306 311

Gly Ile Val Thr Pro
 316

<210> 10
 <211> 341
 <212> PRT
 <213> Candida antarctica

<220>
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 <222> (-24)..(-8)
 <223> secretion signal

<400> 10
 Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 -24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
 -5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
 11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
 26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
 41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
201 206 211 216

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
 251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
 266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
 281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
 301 306 311

Gly Ile Val Thr Pro
 316

<210> 11
 <211> 341.
 <212> PRT
 <213> Candida antarctica

<220>
 <221> SIGNAL
 <222> (-24)..(-1)
 <223> secretion signal

<400> 11
 Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 -24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
 -5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
 11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
201 206 211 216

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe

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<211>	21
<212>	PRT

<113> Artificial Sequence

<240>

<211> a-amylase secretion signal

13

Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala

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10

15

Pro Ala Leu Ala

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<210> 14

<211> 317

<212> PRT

<213> Candida antarctica

<400> 14

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu

1

5

10

15

Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys

20

25

30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe

35

40

45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys

50

55

60

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr

65

70

75

80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn

85

90

95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> LQ53 primer

<400> 15
gctgtgtgtg ggccgcagtt cgtcatcg

28

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LQ35 primer

<400> 16
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30

<210> 17
<211> 30
<212> DNA
<213> Artificial Sequence

<220>

<223> LP53 primer

<400> 17

gtcgccgcgg ctgcgtccc ggcgccggcg

30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

<400> 18

ctgcagccgc cggcgccggg agcgagcc

29